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Original Article

Histopathology of Liver in Diabetes Mellitus - An Autopsy Study

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Abstract

Background: Non-alcoholic steatohepatitis has been a well-known entity in association with Type 2 diabetes mellitus (DM) following insulin resistance and absence of overt hepatic manifestation. We aimed to study the histopathological features of liver in autopsy cases of known DM and identify the significant features and evaluate the extent of hepatic fibrosis seen in DM.

Materials and Methods: Liver histology of 86 autopsy cases of known DM was analyzed over a period of 6½ years with age- and sex-matched controls. Cases with overt hepatic diseases and alcohol intake were excluded from the study. Clinical and biochemical data were acquired from hospital records. Histological features were analyzed using non-alcoholic fatty liver disease scoring system.

Results: Steatosis, lobular and portal inflammation, nuclear glycogenation, and fibrosis were found to be significant features as compared to the controls.

Conclusion: Fibrosis appeared to be the independent feature irrespective of the degree of steatohepatitis, thus contributing to morbidity and mortality among diabetics.

Key words: Autopsy, Liver histopathology, Non-alcoholic fatty liver disease, Type 2 diabetes mellitus

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has been the most common liver disease in the developed world associated with risk factors such as obesity, Type 2 diabetes mellitus (DM), and dyslipidemia, and it is currently emerging as an epidemic in the developing nations as well. Urbanization and changes in lifestyle appear as the key factors for the development of NAFLD/non-alcoholic steatohepatitis (NASH), thus leading to an upsurge in the incidence of this metabolic syndrome. The rates in Asia-pacific region have been estimated to be 12-24% of the general population.[1] Among various factors leading to NAFLD, Type 2 DM appears to be an important factor as there is a rising concern over increasing insulin resistance following chronic liver disease due to NAFLD. In the literature, a high incidence of NAFLD is seen in diabetics, and Type 2 DM appears to be the independent risk factor for NAFLD. The histological spectrum of NAFLD comprises steatosis, steatohepatitis, fibrosis, and cirrhosis; changes resembling like alcoholic liver disease. Hence, the exclusion of history of alcohol consumption is essential for the diagnosis of NAFLD.

Newer imaging modalities and biomarkers are emerging as non-invasive methods for identifying NAFLD. However, histology continues to remain the gold standard for identifying the extent of liver disease. In the literature, this aspect has been assessed mainly on liver biopsies. We aimed to study the liver histology of autopsy cases of known Type 2 DM, identify the significant histological features, and evaluate the extent of fibrosis associated with morbidity and mortality.

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MATERIALS AND METHODS

Autopsy records over a period of 6½ years from January 2001 to June 2007 were reviewed. From a total of 2542

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autopsies, 86 adult autopsies of known Type 2 DM without any obvious liver disease were included in the study. Clinical and biochemical data were obtained from the hospital records. Cases with any previous history of liver disease or alcohol intake were excluded from the study. 55 age- and sex-matched autopsy cases with no history of Type 2 DM or any other liver disease were selected as controls. At least two hematoxylin and eosin (H and E) stained histological sections of liver were studied for both cases and controls.

The histological features of NAFLD identified were based on the NAFLD scoring system as described in the literature. These included grades of steatosis, lobular and portal inflammation, ballooning of hepatocytes, and nuclear glycogenation. Fibrosis was staged from 1 to 4 on the basis of its extent and location, i.e., portal, portal and periportal, bridging, and cirrhosis. Special stains such as Masson trichrome were used to identify stage 1a (pericellular) fibrosis. The results obtained were statistically analyzed using Fisher's test and $P < 0.05$ was considered significant.

RESULTS

Of the 86 autopsy cases with known Type 2 DM reviewed retrospectively for the changes of NAFLD in liver sections, 57 (66.3%) were males and 29 (33.7%) were females. Thus, males outnumbered females with a male:female ratio of 1.9:1. These 86 patients ranged in the age from 33 to 90 years, with mean age being 54.4 years. In majority of the cases, 49 (56.8%) were between 41 and 60 years (Figure 1).

Hepatic Histology

The histological features were identified using the NAFLD scoring guidelines in both cases and controls. Steatosis was seen in 34 (39.5%) cases. Of these, 13 (15.1%), 11 (12.7%), and 10 (11.6%) cases, respectively, showed Grade 1 (5-33%), Grade 2 (33-66%), and Grade 3 (>66%) steatosis. Eight (14.5%) cases out of 55 controls also showed features of steatosis, all of them being of Grade 1.

Lobular inflammation of Grade 1 (<2 foci/×20 field) was observed in majority of 42 (48.8%) cases followed by Grade 0 (no inflammatory foci), Grade 2 (2-4 foci/×20 field), and Grade 3 (>4 foci/×20 field) in 22 (25.5%), 14 (16.2%), and 8 (9.3%) cases, respectively. Portal inflammation was significantly observed in 54 (62.7%) cases, which was Grade 1 (>minimal) as against 32 (37.2%) cases of Grade 0 (none to minimal). The type of inflammation was predominantly of mononuclear cells comprising lymphocytes and plasma cells.

Although ballooning degeneration was observed in only 39 (45.3%) cases, it was still significant as compared to 3 (5.4%) cases among the controls. Nuclear glycogenation of hepatocytes was significantly observed in 59 (68.6%) cases as against none observed in the control cases.

Fibrosis was significantly observed in 34 (39.5%) cases as compared to the controls. Majority of the cases, i.e., 16 (18.6%) showed stage 2 (portal and periportal) fibrosis followed by 10 (11.6%) cases of stage 3 (bridging fibrosis), and 4(4.6%) cases showed stage 4 fibrosis.

Statistical analysis of all the above-mentioned histological features by applying Fisher's test showed them to be significant ($P < 0.05$) in cases studied as compared to the controls (Table 1).

Out of 34 cases of fibrosis, 14 (41.1%) cases showed stage 3 and 4 fibrosis. Majority of these 14 cases had Grade 0 to 1 steatosis and lobular inflammation (Figure 2). Masson's trichrome performed on liver sections did not identify additional cases with fibrosis.

DISCUSSION

The relationship between insulin resistance and liver disease has been well documented in literature.[2,3] Insulin resistance is the central pathogenic mechanism in the development of both Type 2 DM and NASH.[4] Harrison *et al.*[5] have even described a type of diabetic microangiopathy affecting the liver apart from steatohepatitis.

This study was aimed mainly to study liver histopathology in autopsy cases of Type 2 DM and to identify histological

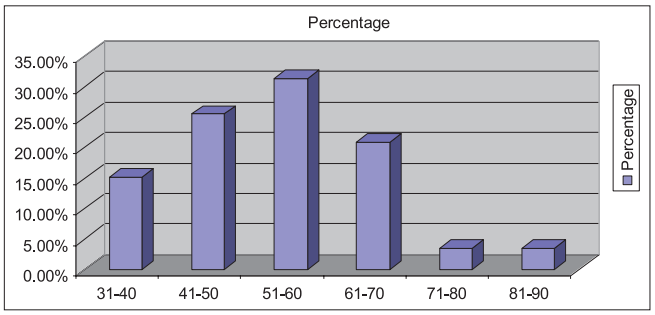

Figure 1: Age distribution of cases

Table 1: Histological features seen in sections from livers of cases

Histological feature	Cases (86) (%)	Controls (55) (%)	*P* value*
Steatosis	34 (39.5)	8 (14.5)	0.023
Lobular Inflammation	64 (74.4)	11 (20)	<0.0001
Portal inflammation	54 (62.7)	11 (20)	<0.0001
Ballooning	39 (45.3)	3 (5.4)	<0.0001
Glycogenated nuclei	59 (68.6)	0	<0.0001
Fibrosis	34 (39.5)	1 (1.8)	<0.0001

*p<0.05

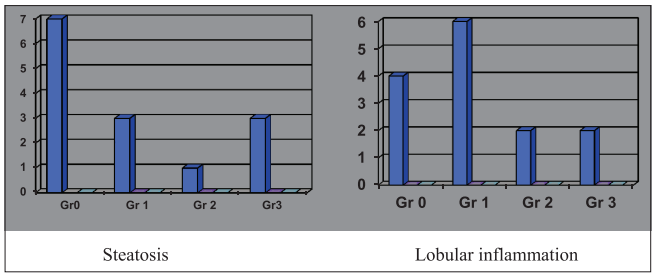

Figure 2: Degree of steatosis and lobular inflammation in cases with stage 3 and 4 fibrosis

features significantly associated with diabetes as compared to non-diabetic controls.

Of the 86 autopsy cases in this study, males outnumbered females with a male:female ratio of 1.9:1. The age range was between 33 and 90 years, with a mean age of 54.4 years.

Gupte *et al.*[6] studied liver biopsies of 32 patients of Type 2 DM for features of NASH. They found that male:female ratio was 3:1 in the group showing only steatosis while it was 1:1.33 in the NASH group. Amarapurkar *et al.*[7] carried out a study with 36 patients of NASH associated with diabetes showing age range of 25-75 years (mean - 50.8 years) and male:female ratio of 1.1:1.

Of the histological features studied, significant steatosis (>5%) was seen in 34 (39.5%) cases as against 8 (14.5%) controls. Most of it was macrovesicular with a variable admixture of microvesicular steatosis. Of these, majority showed Grade 1 steatosis closely followed by Grades 2 and 3. Of 100 patients of Type 2 DM studied by Gupte *et al.*,[6] 49% showed steatosis on ultrasonography and histology. Vasdev *et al.*[8] found that the three grades of steatosis were seen in an equal proportion of patients with NAFLD.

Brunt *et al.*[9] suggested necroinflammatory grading of steatohepatitis into mild, moderate, and severe on the basis of the degree of steatosis, lobular/intra-acinar, and portal inflammation along with hepatocellular ballooning. In the present study, lobular inflammation was seen in 74.4% of the cases as against 20% of the controls ($P < 0.0001$). Of these, majority showed Grade 1 (<2 foci/×20 field) inflammation followed by Grade 2 and Grade 3. Grade 1 (>minimal) portal inflammation was seen in 62.7% of the cases. Portal inflammation was also seen in 20% of the controls, but was mainly Grade 0 (none to minimal). This feature was also found to be significantly more common among the cases ($P < 0.0001$).

Evidence of hepatocyte injury in the form of ballooning degeneration was found in 45.3% of the cases as compared to only 5.4% of the controls. Vasdev *et al.*[8] found evidence of hepatocyte injury in 53.1% of the cases of NAFLD and also in 50% of the cases of chronic hepatitis B and C. Thus, ballooning degeneration, although not specific, along with steatosis and lobular inflammation forms a common set of minimal criteria for the diagnosis of NASH.[10]

Glycogen in the nuclei of human hepatocytes is commonly seen in DM and also in von Gierke's disease, arteriosclerosis, neoplasms, and acute suppurative inflammations. Caramia *et al.*[11] demonstrated various types of nuclear glycogen deposits on electron microscopy in liver biopsies from diabetic patients. In the present study, glycogenated nuclei were seen in 68.6% of the cases as against none in controls. Hence, along with other features of NASH, the presence of glycogenated nuclei suggests the presence of diabetic changes in the liver.

Diabetes, due to the persistence of underlying insulin resistance and abnormalities of fatty acid oxidation, is a known risk factor for the progression of NASH.[12] Fibrosis eventually results due to an increase in the connective tissue and architectural remodeling. At the time of initial presentation, 30-40% of the patients with NASH have advanced fibrosis while 10-15% of the patients have established cirrhosis. In this study, fibrosis was seen in 39.5% of the cases. Majority of these were stage 2 (portal and periportal) and 14 (16.2%) cases showed stage 3 (bridging) and stage 4 (cirrhosis) fibrosis. Vasdev *et al.*[8] found fibrosis in 34.4% of the cases of NAFLD. Gupte *et al.*[6] found fibrosis in 21% of the diabetics and higher degrees of fibrosis in 9.3% of the cases. In their study, they also found that longer duration of diabetes and co-existent risk factors such as obesity potentiate the progression of NASH toward higher degrees of fibrosis. It has been suggested that the presence of pericellular and perivenular fibrosis would point toward advanced disease and the potential for progression. However, this feature was not identified in any additional cases in this study even on Masson's trichrome staining.

A curious feature noted in this study was that of the 14 cases showing stage 3 and 4 fibrosis, majority showed only mild-to-moderate degree of steatosis and lobular inflammation. This finding reiterates the fact that liver histology loses the characteristic markers of the disease as NASH progresses, ending in cirrhosis without specific etiologic features (i.e., cryptogenic cirrhosis). This has been highlighted by Poonawala *et al.*[13] who found that the prevalence of type 2 diabetes was significantly higher in patients with cryptogenic cirrhosis as compared to controls.

To conclude, steatosis, lobular inflammation, portal inflammation, nuclear glycogenation, and fibrosis are the characteristic histopathological changes seen in the liver due to DM. Although these features have been demonstrated in

liver biopsies from electively studied diabetic patients, this study highlights the histopathology of liver in autopsy cases of DM with no ante-mortem evidence of liver disease. In addition, the occurrence of fibrosis was found to be independent of the degree of steatohepatitis in cases of Type 2 DM.

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